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SELECTION OF MONOCLONAL ANTIBODIES

[Selektion von monoklonalen Antikoerpern]

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SELECTION OF MONOCLONAL ANTIBODIES

The invention concerns a process for selecting monoclonal antibodies, which comprises the fusion of B-lymphocytes with myeloma cells to antibody producing hybridoma cells, wherein the antibodies are presented on the cell surface of the hybridoma cells by means of an antibody binding protein, and the binding of the antibodies on antigens. The invention also concerns the media used for this purpose.

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Specification

The invention concerns a process for selecting monoclonal antibodies as well as the media used therefor.

The production of monoclonal antibodies relies on a process developed by Koehler and Milstein. According to this process, B-lymphocytes are fused with myeloma cells, whereby are obtained antibody producing hybridoma cells. Such a process has great advantages. It is particularly complicated to select antibodies, since this requires a separate cultivation of hybridoma cells. The latter causes also that only a limited number of hybridoma cells can be collected and therefore also not all the antibodies can be selected, which is particularly disadvantageous if antibodies with the highest affinity for an antigen should be selected.

It is an object of the invention to make available media with which monoclonal antibodies can be produced, wherein the aforementioned disadvantages are precluded.

This is attained pursuant to the invention with the objects of the patent claims.

The invention is based upon the realization of the applicant that monoclonal antibodies can be presented on the cell surface of hybridoma cells by means of an antibody binding protein. It was realized that monoclonal antibodies could be selected herewith without having to cultivate hybridoma cells separately. It was also realized that the selection of monoclonal antibodies can occur also with a view to their affinity strength to specific antigens.

The realizations of the applicant were utilized pursuant to the invention to make available a process for selecting monoclonal antibodies. Such a process comprises the fusion of B-lymphocytes with myeloma cells to antibody producing hybridoma cells, wherein the antibodies are presented on the cell surface of the hybridoma cells by means of an antibody binding protein, and the binding of the antibodies to antigens.

The term "B-lymphocytes" comprises B-lymphocytes of any kind and origin. They can also be preliminary stages of B-lymphocytes. The B-lymphocytes can furthermore originate from

animals, such as mice, rats, rabbits, et cetera, or from humans. The B-lymphocytes can likewise originate from a healthy or sick organism. It is advantageous if they originate from an immunized organism. It is particularly advantageous if the B-lymphocytes code for human antibodies or parts thereof. If they are B-lymphocytes of animals, this can be achieved in that the animals are transgenic for human antibodies or parts thereof. The production of these animals can occur through the usual processes, wherein it is offered to introduce the genes for human antibodies or parts thereof into embryonic strain cells, from which the animals will be generated. The provision of B-lymphocytes and their preliminary stages can occur through the usual processes.

The term "myeloma cells" comprises myeloma cells of any type and origin. They can also be precursors of myeloma cells.

The myeloma cells can furthermore originate from animals, such as mice, rats, rabbits, et cetera, or from humans. The B-lymphocytes can likewise originate from a healthy or sick organism. The preferred myeloma cells are descendants of the mouse strains P3K, P3-X63.Ag8, X63.Ag8.653, NSO/1, Sp2/O-Ag14, and F0, the rat strains Y3-Ag.1.2.3, YB2/0, and IR9834, and the human strains U266, SK007, and Karpas 707. The provision of

myeloma cells and their preliminary stages can occur through the usual processes.

The term "antibody producing hybridoma cells" comprises cells that are produced by fusing B-lymphocytes and myeloma cells and that produce antibodies. It is referred herein to the corresponding embodiments concerning B-lymphocytes and myeloma cells. The hybridoma cells can have animal and/or human nucleic acids or proteins. The cultivation of hybridoma cells can occur through the usual processes. It can be likewise advantageous if the hybridoma cells (over)express recombinases, for example, Rag1 or Rag2, and/or mutases. This can be attained through transfection of hybridoma cells with corresponding expression vectors. The person skilled in the art has knowledge of these expression vectors.

The term "fusion of B-lymphocytes with myeloma cells" concerns any process with which these cells can be fused. An advantageous embodiment in which the cells are fused over polyethylene glycol is advantageous. Reference is made herein to the examples.

The term "binding of the antibodies on an antigen" concerns any possible process with which the antibodies expressed on the surface of the hybridoma cells can bind on the antigens. The antigens can be bound on substrates, for example, magnetic

beads. They can also be marked, for example, fluorescence marked. As fluorescent marker are offered, for example, FITC, TRITC, Cy3, Cy5, Cy5.5, Cy7, and phycoerythrin. The antigens can furthermore be coupled to biotin. The bound antibodies can then be detected through the usual processes, for example, FACS analysis, whereby also the corresponding antibodies are detected. Reference is made herein to the examples.

The term "antibody binding protein" comprises any protein that can bind an antibody and can present the same on the cell surface of hybridoma cells. The protein can have in particular a signal peptide, an antibody binding point independent from the specialty of the antibody, and a membrane anchor. Examples of such a protein are natural Fc binding proteins, such as CD16, CD32, and CD64. Furthermore, the proteins can have a combination of a signal peptide, an antibody binding point, and a membrane anchor, which does not occur in nature. Such a combination can comprise parts of natural Fc binding proteins. It can also have as signal peptide one of a mouse Ig kappa chain or a mouse MHC class I k(k) molecule, as membrane anchor a transmembrane domain of PDGRF or CD52, and as antibody binding point an antigen binding domain of a bacterial protein, such as protein A, protein G, protein L, or protein LG. It may be advantageous if the combination has several signal peptides,

antibody binding cells, and/or membrane anchors. It may be particularly advantageous if the antibody binding protein, in particular the antibody binding domain of the bacterial proteins, has codons that are optimized for the expression in mammal cells. The person skilled in the art knows which codons these are.

Preferred antibody binding proteins are disclosed in Figs. 1-3. The antibody binding protein of Fig. 1 comprises the signal peptide of a mouse MHC class I k(k), four antibody binding domains of protein L, and the transmembrane domain of CD52. The DNA and amino acid sequences of the antibody binding protein are disclosed between the nucleotide numbers 682-1782. The antibody binding protein of Fig. 2 comprises the signal peptide of a /3 mouse Ig kappa chain, two antibody binding points of protein G, and the transmembrane domain of CD52. The DNA and amino acid sequences of the antibody binding protein are disclosed between the nucleotide numbers 647-1420. The antibody binding protein of Fig. 3 comprises the signal peptide of the mouse MHC class I k(k) molecule, two antibody binding points of protein G, and the transmembrane domain of PDGFR. The DNA and amino acid sequences of the antibody binding protein are disclosed between the nucleotide numbers 682-1431. The antibody binding points of all

three antibody binding proteins have codons on the DNA plane, which are optimized for the expression in mammal cells.

An antibody binding protein of Fig. 1, 2, or 3 can have an amino acid sequence that differs by one or several amino acids from the amino acid sequence in Fig. 1, 2, or 3. The differences can be related to additions, deletions, substitutions, and/or inversions of individual amino acids. The DNA hybridizes this antibody binding protein with the DNA disclosed in Fig. 1, 2, or 3. The term "hybridization" indicates a hybridization under customary conditions, in particular at 20°C below the melting point of DNA. The antibody binding protein with the modified amino acid sequences has also total and partial functions that are comparable to those of the antibody binding proteins of Fig. 1, 2, or 3.

A further object of the invention is a nucleic acid, which codes for a preceding antibody binding protein. The nucleic acid can be an RNA or a DNA. Preferred is a DNA, comprising the following:

(a) the DNA of an antibody binding protein of Fig. 1, 2, or 3, a DNA differing therefrom by one or several base pairs, or

(b) a DNA related to the DNA of (a) via the degenerated genetic code.

The term "a DNA differing by one or several base pairs" comprises any DNA coding for an antibody binding protein of Fig. 1, 2, or 3, which hybridizes with the DNA of Fig. 1, 2, or 3. The differences can be related to additions, deletions, substitutions, and/or inversions of individual base pairs. Concerning the term "hybridization," it is referred to the preceding explanations.

A DNA pursuant to the invention can be available as such or in combination with any other DNA. A DNA pursuant to the invention coding for an antibody binding protein can be present in particular in an expression vector. Examples of these are known to persons skilled in the art. In the case of an expression vector for E. coli, these can be, for example, pGEMEX, pUC derivatives, pGEX-2T, pET3b, and pQE-8. For the expression in yeast should be mentioned, for example, pY100 and Ycpad1, while for the expression in animal cells are disclosed, for example, pKCR, pEFBOS, pCDM8, and pCEV4. For the expression in insect cells is particularly suitable the Bacculovirus expression vector pAcSGHisNT-A.

The person skilled in the art knows in which way the DNA pursuant to the invention must be inserted into the expression vector. The person skilled in the art knows also that these DNAs can be inserted in connection with a DNA coding for another

protein or peptide, so that the DNA pursuant to the invention can be expressed in the form of a fusion protein.

Preferred expression vectors that contain a DNA pursuant to the invention are disclosed in Figs. 1-3. These are the expression vectors pSEX11L4, pSEX11G2*, and pSEX15G2. These were deposited at the DSMZ (German Collection of Microorganisms and Cell Cultures) on December 14, 1998. In detail, PSEX11L4 was deposited under DSM 12580, pSEX11G2* was deposited under DSM 12581, and pSEX15G2 was deposited under DSM 12582.

The person skilled in the art knows suitable cells for the expression of the DNA pursuant to the invention available in an expression vector. Examples of such cells comprise the E. coli strains XL-1 blue, Top 10 F, HB101, DHSalpha, x1776, JM101, JM109, BL21, and SG13009, the yeast strains Saccharomyces cerevisiae and Pichia pastoris, the animal cells L, NIH 3T3, FM3A, CHO, COS, Vero, HeLa, myeloma and hybridoma cells, as well as insect cells sf9.

The person skilled in the art knows furthermore about the conditions required for the cultivation of transformed or transfixed cells. The person skilled in the art is also aware of processes that allow the isolation and purification of protein or fusion protein through the DNA expressed pursuant to the invention.

A further object of the invention is an antibody directed against an aforementioned protein or fusion protein. Such an antibody can be produced through the usual processes. It can be polyclonal or monoclonal. It is advantageous for its production to immunize animals, in particular rabbits or chickens, for a polyclonal antibody, and mice for a monoclonal antibody, with an aforementioned (fusion) protein or fragments thereof. Further "booster injections" of the animals can occur with the same (fusion) protein or fragments thereof. The polyclonal antibody can be obtained then from the serum or egg of the animals. For the monoclonal antibody are fused spleen cells of animals with myeloma cells.

A further object of the invention is a kit. Such a kit comprises one or several of the following components:

(a) a DNA pursuant to the invention,

(b) a cell that expresses a DNA pursuant to the invention,

(c) an antibody binding protein pursuant to the invention,

(d) an antibody pursuant to the invention, as well as

(e) customary additives, such as substrates, puffers, solvents, controls, markers, and detection reagents for the components (a)-(d).

Of the individual components can be available respectively one or several representatives. With regard to the individual

terms, it is referred to the aforementioned explanations. These apply accordingly herein.

The invention is characterized in that antibodies produced by hybridoma cells are presented on the cell surface of hybridoma cells. This occurs via an antibody binding protein. Such antibody binding protein can be introduced into the hybridoma cells in order to produce the myeloma cells used for the production of hybridoma cells. The antibody binding protein can also be introduced into the hybridoma cells via an expression vector coding therefor.

With the invention, it is possible to select antibodies. This can occur without great effort, since the hybridoma cells do not have to be cultivated separately. Rather, complex mixtures of hybridoma cells can be utilized directly for the selection of antibodies. The antibodies can furthermore be selected in accordance with their affinity strength to specific antigens. /4 The invention is furthermore suitable for selecting antibodies of hybridoma cell libraries not only with respect to a specific antigen, but also with respect to many (un)determined antigens of antigen libraries.

The invention supplies thus a medium with which, among other things, the great time and cost problems can be precluded, which occurred until now in the selection of monoclonal antibodies.

Brief Description of the Drawings

Fig. 1 shows the expression vector PSEX11L4 pursuant to the invention (Fig. 1(A)), which codes for an antibody binding protein (Fig. 1(B)). Reference is made to the preceding explanations.

Fig. 2 shows the expression vector pSEX11G2 pursuant to the invention (Fig. 2(A)), which codes for an antibody binding protein (Fig. 2(B)). Reference is made to the preceding explanations.

Fig. 3 shows the expression vector pSEX15G2 pursuant to the invention (Fig. 3(A)), which codes for an antibody binding protein (Fig. 3(B)). Reference is made to the preceding explanations.

The invention will be explained based on the following examples.

Example 1

Production of Myeloma Cells that Express an Antibody Binding Protein on their Cell Surface

(A) Transient Expression

Cells of the myeloma cell line X63-Ag8.653 are used. These cells (10^7) are transfected with 20-40 µg of the expression vector pSEX11G2* pursuant to the invention (see Fig. 2). As transfection technique is carried out an electroporation, which

comprises two pulses of 500 V for 2 ms. The cells are incubated for 48 hours in RPMI medium containing 0% of FCS, at 37°C and 5-7.5% of CO_2. Thereafter, the cells are washed with cold DPBS + 0.1% sodium azide before they are incubated for 45 minutes at 0°C with DPBS + 0.1% sodium azide plus 25 µg/ml of goat anti calf antibody (FITC marked; GAB-FITC, Dianova). After washing with DPBS + 0.1% sodium azide, the cells are incubated with DPBS + 0.1% sodium azide + 1 µg/ml of propidium iodide, and subjected to a FACS analysis after being excited with blue light.

It is shown that the transfected myeloma cells have a green fluorescence, which is induced by the transient expression of an antibody binding protein on the cell surface of the myeloma cells.

(B) Stable Expression

The myeloma cells obtained under (A) are subjected to a G418 selection lasting 14-24 days before they are incubated with GAB-FITC and subjected to a FACS analysis, as described under (A). The myeloma cells, which have a strong green fluorescence, are subjected to further G418 selection rounds.

The myeloma cell line X63-Ag8.653.3 is obtained, which stably expresses an antibody binding protein on its cell surface.

Example 2

Production of Hybridoma Cells that Can Express Antibodies on their Cell Surface by Means of an Antibody Binding Protein

(A) 10 balb/c mice are subcutaneously immunized with 100 µg of killed Helicobacter pylori bacteria in complete Freund's adjuvant, which contains killed myelobacter tuberculosis bacteria. After 4 or 7 weeks occurs respectively an intraperitoneal booster injection with 100 µg killed Helicobacter pylori/Mycobacter tuberculosis bacteria. 100 µg of blood serum are extracted from the mice before each immunization or after the last immunization, respectively, and the antigen specific immune response of the mouse is checked in western blot. As antigen is used a decomposition of bacterial total protein of Helicobacter pylori or Mycobacter tuberculosis. The detection of bound mouse antibody is conducted through a peroxidase conjugated goat anti mouse antibody (Dianova). The spleen of mice with a clear antigen specific immune response is removed and the lymphocytes are fused with cells of the myeloma cell line X63-Ag8.653.3 of Example 1(B). The fusion occurs through polyethylene glycol (see Goding, J.W., Cell Biology, Biochemistry and Immunology, 3rd edition (1996), Academic Press Limited Publishers, 24-28). Hybridoma cells are obtained.

These are incubated for 10-12 days in HAT medium at 37°C. The hybridoma cell library 2A is obtained.

Hexapeptides with N-terminated biotin are synthesized. The peptides correspond to the 6C-terminated amino acids of 101 or 118 gene products of Helicobacter pylori or Mycobacter tuberculosis. 103 cells of the hybridoma cell library 2A are likewise washed with cold DPBS + 0.1% sodium azide and incubated for 45 minutes at 0°C with DPBS + 0.1% sodium azide + 10 µg of the aforementioned biotin marked peptides. The cells are washed with cold DPBS + 0.1% sodium azide and incubated for 45 minutes at 0°C with 10 µg/ml of streptavidin FITC. After washing with DPBS + 0.1% sodium azide, the cells are incubated in DPBS + 0.1% sodium azide + 1 µg/ml of propidium iodide and subjected to an FACS analysis after excitation with blue light.

It is shown that the hybridoma cells have a green fluorescence. This fluorescence is induced by the expression of antibodies on the cell surface of the hybridoma cells. Continuing tests show that the antibodies have an anti Helicobater pylori or Mycobacter tuberculosis activity.

(B) Cells of the hybridoma cell line U98/6, which produce a mouse anti urokinase antibody, are used. These cells (10^7) are transfected with 20-40 µg of the expression vector pSEX11G2*

pursuant to the invention (see Fig. 2). As transfection technique is carried out an electroporation, which comprises two pulses of 400 V for 2 ms. The cells are incubated for 48 hours in an incomplete AIM V medium at 37°C and 5-7.5% of CO_2. Thereafter, the cells are washed with cold DPBS + 0.1% sodium azide before they are incubated for 45 minutes at 0°C with DPBS + 0.1% sodium azide + 10 µg/ml of urokinase biotin. After washing with DPBS + 0.1% sodium azide, the cells are incubated with DPBS + 0.1% sodium azide + 10 µg/ml of streptavidin FITC, and subjected to a FACS analysis after being excited with blue light.

It is shown that the transfected hybridoma cells have a green fluorescence. This fluorescence is induced by the expression of antibodies on the cell surface of the hybridoma cells. Continuing tests show that the antibodies have an anti urokinase/5 activity.

The obtained hybridoma cells are subjected to a 14-24 day G418 selection before they are again incubated, as described above, with urokinase biotin and streptavidin FICS and subjected to a FACS analysis. The hybridoma cells, which have a strong green fluorescence, are subjected to further G418 selection rounds.

The hybridoma cell line U98/6.3.3 is obtained, which stably expresses antibodies on its cells surface.

Example 3

Selection of Monoclonal Antibodies that Are Expressed by Means of an Antibody Binding Protein on the Cell Surface of Hybridoma Cells

10^3 cells of the hybridoma cell line U98/6.3.3 of Example 2(B) are mixed with 10^7 cells of the hybridoma cell line DOB.L1.3. The latter hybridoma cell line produces an antibody that detects the C-terminus of the human HLA-DO-β chain. The latter is expressed on the cell surface by means of the same antibody binding protein as in the hybridoma cell line U98/6.3.3 of Example 2(B). The cell mixture is washed with cold DPBS + 0.1% sodium azide and incubated for 45 minutes at 0°C with DPBS + 0.1% sodium azide + 10 µg/ml of urokinase biotin. After washing with DPBS + 0.1% sodium azide, the cell mixture is incubated with DPBS + 0.1% sodium azide + 10 µg/ml of streptavidin FITC, and subjected to a FACS sorter after being excited with blue light.

Hybridoma cells having a green fluorescence are selected. In continuing tests, they show an anti urokinase activity. The hybridoma cells U98/6.3.3 51-S50 are obtained.

Example 4

Production and Purification of an Antibody Binding Protein Pursuant to the Invention

(A) The DNA of Fig. 1 between the nucleotide numbers 682-1782 is provided with BAMHI links, split with BamHI, and inserted into the split expression vector pQE-8 (Qiagen) with the BamH. The expression of plasmid pQE-8/antibody binding protein is achieved. One such as this codes for a fusion protein of 6 histidine radicals (N-terminated partner) and the antibody binding protein of Fig. 1 (C-terminated partner) pursuant to the invention. pQE-8/antibody binding protein is used for the transformation of E. coli SG 13009 (see Gottesman, S. et al, J. Bacteriol. 148 (1981), 265-273). The bacteria are cultivated in an LB medium with 100 µg/ml of ampicillin and 25 µg/ml of kanamycin and induced for 4 hours with 60 µM of isopropyl-β-D thiogalactopyranoside (IPTG). By adding 6 M of guanidine hydrochloride is achieved a lysis of bacteria, and a chromatography (Ni-NTA resin) is carried out in addition with the lysate in the presence of 8 M urea corresponding to the data of the manufacturer (Qiagen) of the chromatography material. The bound fusion protein is eluted in a puffer with pH 3.5. After its neutralization, the fusion protein is subjected to an 18% SDS-polyacrylamide gel electrophoresis and dyed with

Coomassie blue (see Thomas, J.O. and Kornberg, R.D., J. Mol. Biol. 149 (1975), 709-733).

It is shown that the antibody binding protein (fusion protein) can be produced in a highly pure form.

(B) 10^8 cells of the myeloma cell line X63-Ag8.653.3 of Example 1(B) are washed with PBS and absorbed into PBS + 1% Tween 20, and incubated on ice. Partial cell components are separated by centrifugation at 30,000 g and the residue is poured on an IgG sepharose column (IgG Sepharose 6 Fast Flow Lab Pack of Pharmacia). Unbound components are removed by washing, and the antibody binding protein pursuant to the invention is eluted in acid pH.

After its neutralization, the antibody binding protein is subjected to an 18% SDS polyacrylamide gel electrophoresis and dyed with Coomassie blue (see above).

It was shown that an antibody binding protein pursuant to the invention (fusion protein) can be produced in a highly pure form.

Example 5

Production and Detection of an Antibody Pursuant to the Invention

A fusion protein pursuant to the invention of Example 4 is subjected to an 18% SDS polyacrylamide gel electrophoresis.

After dyeing the gel with 4 M sodium acetate, an approx. 41 kD strip is cut out of the gel and incubated in a cooking salt solution buffered in phosphate. Gel pieces are sedimented before the protein concentration of the residue is determined by means of an SDS polyacrylamide gel electrophoresis, which is followed by a Coomassie blue dyeing. Animals are immunized as follows with the gel-purified fusion protein:

Immunization Schedule for Polyclonal Antibodies in Rabbits

Per immunization are used 35 µg of purified fusion protein in 0.7 ml of PBS and 0.7 ml of complete or incomplete Freund's adjuvant.

Day 0: 1st immunization (complete Freund's adjuvant)

Day 14: 2nd immunization (incomplete Freund's adjuvant; icFA)

Day 28: 3rd immunization (IcFA)

Day 56: 4th immunization (IcFA)

Day 80: Bleeding

The rabbit serum is tested in Immunoblot. For this purpose, a fusion protein pursuant to the invention of Example 4 is subjected to an SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose filter (see Khyse-Andersen, J., J. Biochem. Biophys. Meth. 10 (1984), 203-209). The western blot analysis was carried out as described in Bock, C.-T. et al, Virus Genes 8 (1994), 215-229). For this purpose, the

nitrocellulose filter was incubated for one hour at 37°C with a first antibody. This antibody is the rabbit serum (1:1000 in PBS). After several washing steps with PBS, the nitrocellulose filter is incubated with a second antibody. This antibody is a monoclonal goat anti rabbit IgG antibody (Dianova) coupled with alkaline phosphatase (1:5000) in PBS. After a 30-minute incubation at 37°C follow several washing steps with PBS and then the alkaline phosphatase detection reaction with developer solution (36 µM of 5'-bromo-4-chloro-3-indolyl phosphate, 400 µM of Nitroblue tetrazolium, 100 mM of tris-HCl, 100 mM of NaCl, 5 mM of $MgCl_2$) at ambient temperature until bands become visible. /6

It is shown that polyclonal antibodies can be produced pursuant to the invention.

Immunization Schedule for Polyclonal Antibodies in Chickens

Per immunization are used 40 µg of purified fusion protein in 0.8 ml of PBS and 0.8 ml of complete or incomplete Freund's adjuvant.

Day 0: 1st immunization (complete Freund's adjuvant)

Day 28: 2nd immunization (incomplete Freund's adjuvant; icFA)

Day 50: 3rd immunization (IcFA)

Antibodies are extracted from the egg yolk and tested in western blot. Polyclonal antibodies pursuant to the invention are detected.

Immunization Schedule for Monoclonal Antibodies of Mice

Per immunization are used 12 µg of purified fusion protein in 025 ml of PBS and 0.25 ml of complete or incomplete Freund's adjuvant; in the 4th immunization, the fusion protein is dissolved in 0.5 ml (without adjuvant).

Day 0: 1st immunization (complete Freund's adjuvant)

Day 28: 2nd immunization (incomplete Freund's adjuvant; icFA)

Day 56: 3rd immunization (IcFA)

Day 84: 4th immunization (PBS)

Day 87: Fusion

Residues of hybridomas are tested in western blot. Monoclonal antibodies pursuant to the invention are detected.

Patent Claims

1. A process for selecting monoclonal antibodies, comprising the fusion of B-lymphocytes with myeloma cells to antibody producing hybridoma cells, wherein the antibodies are presented on the cell surface of the hybridoma cells by means of an antibody binding protein, and the binding of the antibodies on antigens.

2. The process of claim 1, wherein the antibody binding protein comprises a signal peptide, an antibody binding point dependent from the specificity of the antibody, and a membrane anchor.

3. The process of claim 2, wherein the antibody binding protein comprises a Fc binding protein or parts thereof.

4. The process of claim 2, wherein the antibody binding protein comprises a combination of Fc binding proteins or parts thereof.

5. The process of claim 3 or 4, wherein the Fc binding protein is CD16, CD32, or CD 64.

6. The process of one of the claims 2-5, wherein the antibody binding protein comprises an antibody binding domain of the proteins A, G, L, or LG.

7. The process of claim 2, wherein the antibody binding protein comprises a combination of the signal peptide of a mouse Ig kappa chain or a mouse MHC class I k(k) molecule, an antibody binding point of the proteins A, G, L, or LG, and the transmembrane domain of PDGFR or CD52.

8. The process of claim 7, wherein the antibody binding protein is that of Figs. 1, 2, or 3.

9. The process of one of the claims 1-8, wherein the hybridoma cells (over)express Rag 1 and/or Rag 2.

10. The process of one of the claims 1-9, wherein the antigens originate from an antigen library.

11. The process of one of the claims 1-10, wherein the antigens are bound on a substrate.

12. The process of claim 11, wherein the substrate comprises magnetic beads.

13. The process of one of the claims 1-10, wherein the antigen comprises a fluorescent or biotin marking.

14. The process of claim 13, wherein the fluorescent marking comprises FITC, TRITC, Cy3, Cy5, Cy5.5, Cy7, and phycoerythrin.

15. An antibody binding protein, wherein the antibody binding protein comprises a combination of the signal peptide of a mouse Ig kappa chain or a mouse MHC class I k(k) molecule, an antibody binding point of the proteins A, G, L, or LG, and the transmembrane domain of PDGFR or CD52.

16. The antibody binding protein of claim 15, wherein the antibody binding protein comprises the amino acid sequence of Figs. 1, 2, or 3, or a amino acid sequence that differs therefrom via one or several amino acids.

17. A DNA coding for the antibody binding protein of claim 16, comprising:

(a) the DNA of an antibody binding protein of Fig. 1, 2, or 3, a DNA differing therefrom by one or several base pairs, or

(b) a DNA related to the DNA of (a) via the degenerated genetic code.

18. An expression vector coding for the DNA of claim 17.

19. Cells containing the expression vector of claim 18.

20. An antibodies directed against the antibody binding protein of claim 15 or 16.

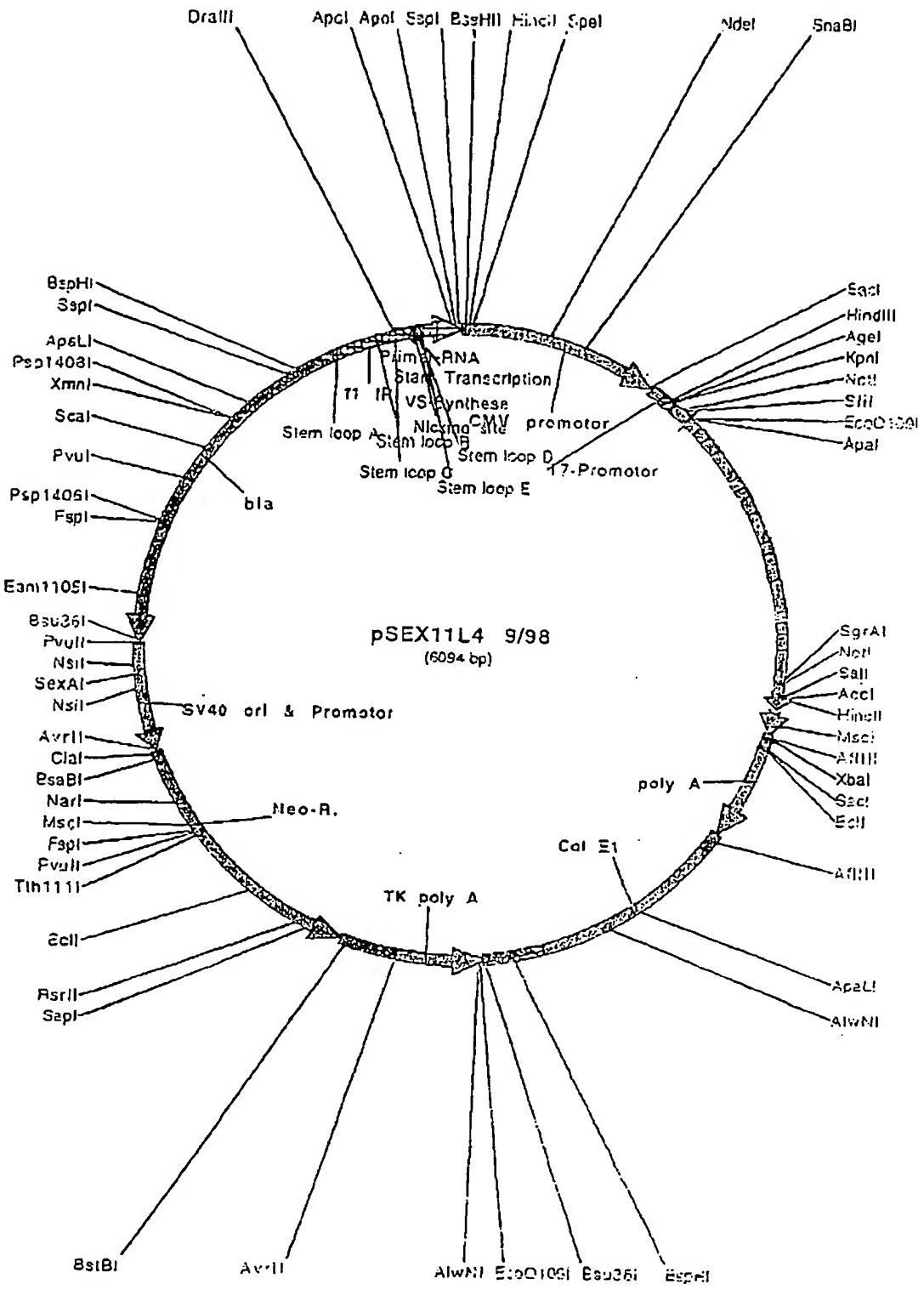
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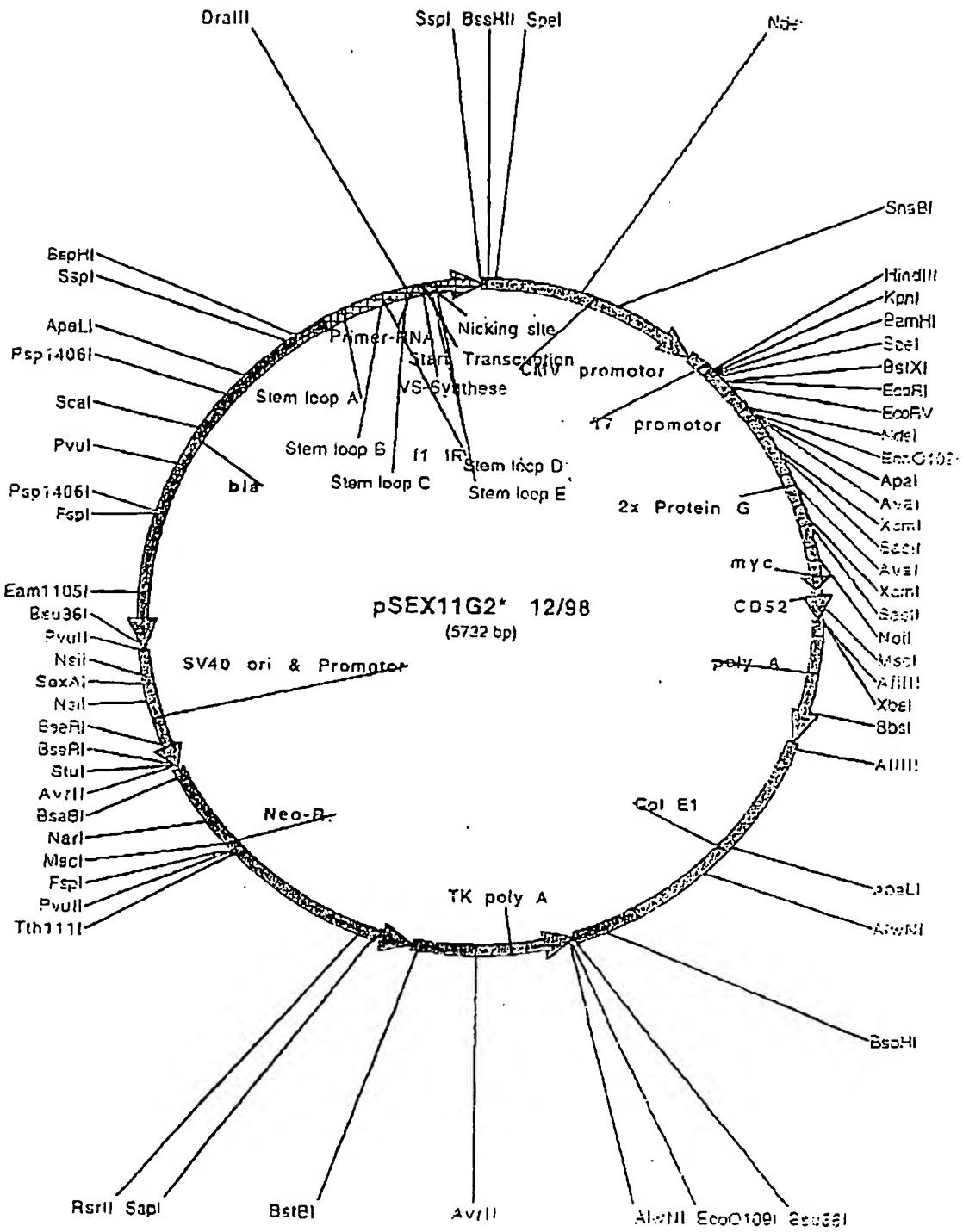
Fig. 1 (A)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NarI
MscI
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BamHI
SceI
BsiXI
EcoRI
EcoRV
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BstBI
AvrII
AlwNI EcoO109I Bsu36I
Primer-RNA
Nicking site
Start Transcription
VS-Synthese
Stem loop A
Stem loop B
Stem loop C
f1 IR
Stem loop D
Stem loop E
CMV promotor
T7 promotor
2x Protein G
myc
CDS2
poly A
SV40 ori & Promotor
bla
Neo-R
TK poly A
pSEX11G2* 12/98
(5732 bp)

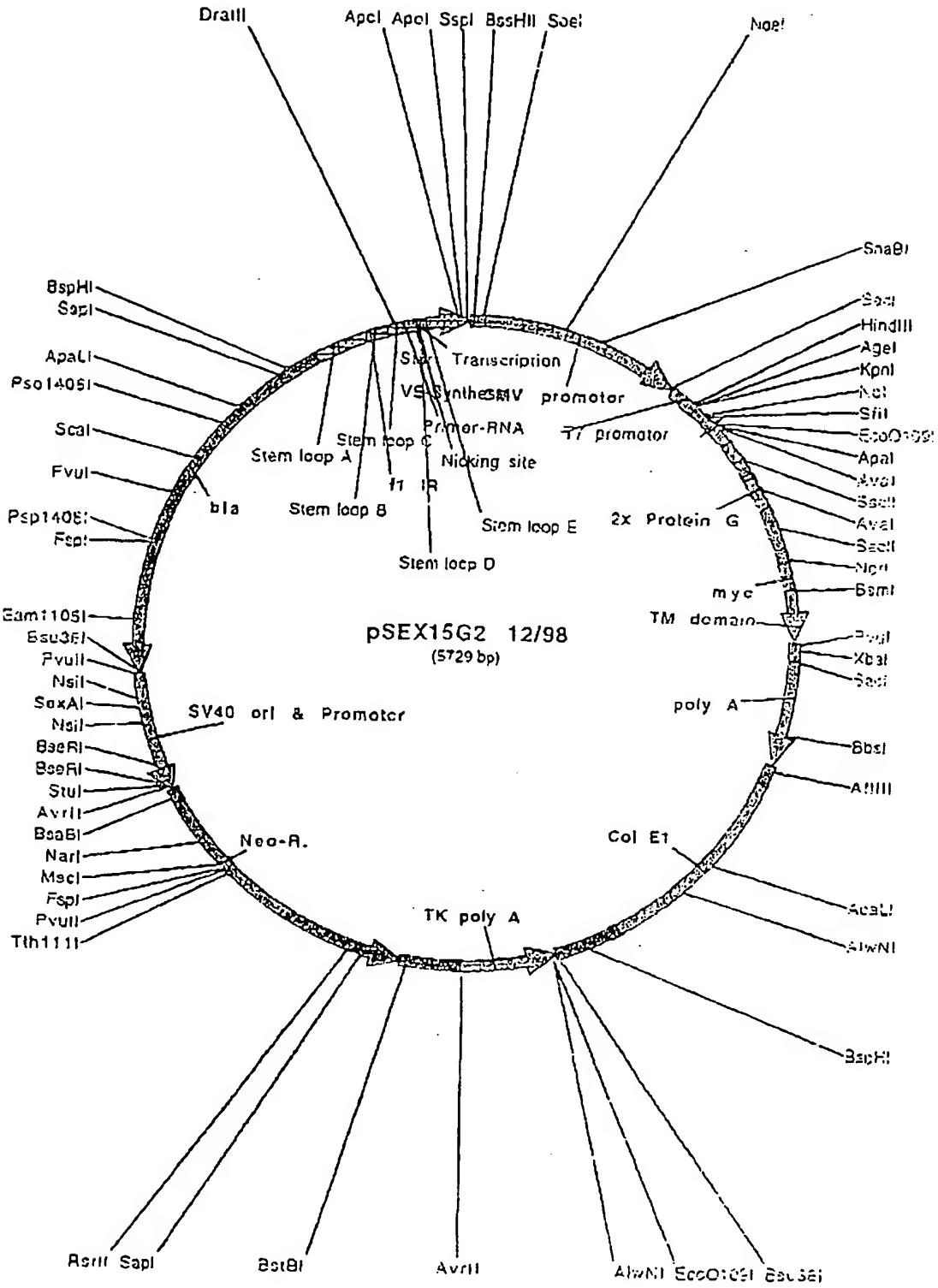
DraIII
ApaI ApoI SspI BssHII SacI
NcoI
SnaBI
SacI
HindIII
AgeI
KpnI
NotI
SfiI
EcoO109I
ApaI
AvaI
SacII
AvaI
SacII
NcoI
BamHI
XbaI
SacI
BbsI
AflIII
AcaCI
AlwNI
BspHI
SapI
ApaLI
Pso1405I
ScaI
FvuI
Psp1406I
FspI
Eam1105I
Bsu36I
PvuII
NsiI
SexAI
NsiI
BseRI
BseRI
StuI
AvrII
BsaBI
NarI
MscI
FspI
PvuII
Tth111I
AsrII SapI
BstBI
AvrII
AlwNI EcoO109I Bsu36I
BspHI
Transcription
SV40 promotor
T7 promotor
Primer-RNA
Nicking site
Stem loop A
Stem loop B
Stem loop C
f1 IR
Stem loop D
Stem loop E
2x Protein G
myc
TM domain
poly A
Col E1
TK poly A
Neo-R.
SV40 ori & Promoter
bla
pSEX15G2 12/98
(5729 bp)

Fig. 3 (A)